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Retention of Vitality by Dried Fruit-Bodies of Certain Hymenomycetes

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UPON THE RETENTION OF VITALITY BY DRIED FRUIT-BODIES OF CERTAIN HYMENOMYCETES INCLUDING AN ACCOUNT OF AN EXPERIMENT WITH LIQUID AIR.

By Professor A. H. R. Buller, D.Sc., Ph.D., F.R.S.C.

In 1909 it was shown by the writer* that the fruit-bodies of a considerable number of species of Hymenomycetes which grow on sticks and logs, can be dried up by exposure to the air in rooms without any loss of vitality, and that the vitality of some species, after desiccation has taken place, may be continued for years. When the fruit-bodies are moistened once more by placing wet cotton-wool on their upper surfaces, after a few hours, spore-liberation recommences. A stream† of spores is emitted for several days, and the spores liberated from the revived fruit-bodies are capable of germination. Typical xylophilous genera having fruit-bodies, the vitality of which will resist desiccation, are: Corticium, Stereum, Phlebia, Daedalea, Polystictus, Schizophyllum, and Lenzites. Many species of Marasmius and some species of Collybia, the mycelium of which vegetates in turf or among dead leaves, also have fruit-bodies which retain their vitality when they have been dried.

Further investigations‡ made it clear that the dried fruit-bodies, when exposed to the air, lose their vitality in the course of a few months or years in the same manner as the seeds of Flowering Plants. Thus *Marasmius oreades* recovered on moistening after desiccation for six weeks but not after three months, and *Lenzites betulina* recovered after desiccation for three years but not after five years.

Since the publication of my "Researches on Fungi," I have carried out some further investigations upon the vitality of the fruit-bodies of Hymenomycetes. A short account will here be given of some of the results of the work.

A number of additional species have been tested for the re-

* Buller, "Researches on Fungi," Longmans, Green & Co., London, 1909, pp. 105-111.

† The emission of spores from fruit-bodies can be seen readily by my beam-of-light method. *Ibid.*, pp. 94-101.

‡ Buller, "Researches on Fungi," p. 111.

tention of the vitality of the fruit-bodies in the dried condition. Successful retention was found for *Panus stipticus, Claudopus (Pleurotus) nidulans, Polyporus brumalis, Exidia glandulosa, Hirneola Auricula-Judae, Calocera cornea,* and the younger fruit-bodies of *Collybia velutipes.*

It is quite certain that the mature fruit-bodies of Coprinus, Bolbitius, Panaeolus, Stropharia, as well as those of a number of other fleshy genera of Agaricineae, will not survive desiccation. However, very young fruit-bodies may behave in an entirely different manner, as is shown by the following observations upon species of the delicate genus Coprinus. At Banbury, England, in the month of August, I found some young and unexpanded fruit-bodies of *Coprinus niveus* coming up on some horse dung in a field. I collected the dung, allowed it to dry by exposing it on a table in a dry room, and subsequently took it to Winnipeg. In the following April, eight months after the material had been dried, it was set in a damp-chamber and moistened. The young fruit-bodies immediately revived and were seen as small snow-white balls about 2 mm. in diameter. On the night of the fourth day after revival, the stipes elongated, the pilei underwent their usual process of autodigestion, and millions of spores were liberated. Similar observations have been made on other species of Coprinus: *C. ephemerus, C. bisporigera,** etc. In these cases the young fruit-bodies, although unexpanded, had their pileus, stipe, and gills differentiated from one another before desiccation took place.

Falck† pointed out that the mycelium of *Coprinus sterquilinus* is still able to continue its development after the horse-dung balls in which it has existed have been kept dry for a year. Observations of my own on a considerable number of species of Coprinus have convinced me that the retention of vitality by the dried mycelium for some months or years is the rule in this genus. The young fruit-bodies of the Coprini, therefore, share their resistance to desiccation with the mycelium from which they spring.

Fruit-bodies of *Daedalea unicolor* can retain their vitality kept in the dark, and exposed to ordinary air at room temperatures, for at least eight years and three months, and those of *Schizophyllum commune* for at least six years and three months. These two species retain their vitality longer than any others which have been tested. In most species, e.g., *Polystictus versicolor, Lenzites betulina,* and *Phlebia pileata,* the vitality persists only for two or three years and is lost in less than five.

* A new species having basidia which bear only two spores. *Vide* Buller, The Production and Liberation of Spores in the genus Coprinus. Trans. Brit. Myc. Soc. 1911, p. 350.

† R. Falck, Beiträge zur Biologie der Pflanzen, Bd. viii., 1902, p. 317.

Fruit-bodies of *Daedalea unicolor* which have been kept dry for eight years and three months, and of *Schizophyllum commune* which have been kept dry for six years and three months, revive much more slowly than fruit-bodies only one year old, shed a reduced number of spores, and become putrid relatively sooner. It is evident that their vitality has become very much diminished and that shortly it will be extinguished altogether. Possibly the vitality of dried fruit-bodies does not persist in any species of Hymenomycetes longer than ten years, but only further experiment can settle this question. The following Table contains the experimental results for a few of the species tested:—

LIST OF SOME HYMENOMYCETES WITH FRUIT-BODIES WHICH CAN BECOME DESICCATED WITHOUT LOSING THEIR VITALITY.

Family.	Species.	Recovered after dessication for:	Failed to recover after dessication for:
Thelephoreae	Stereum purpureum ...	1 year	5 years 3 months
	„ bicolor ...	1 year 6 months ...	2 years
Hydneae	Phlebia pileata ...	2 years 8 months ...	4 years 4 months
	„ zonata ...	1 year	3 years 6 months
Polyporeae	Merulius corium ...	2 years	5 years 3 months
	Daedalea unicolor ...	8 years 3 months ...	———
	Polyporus rigens ...	2 years	6 years 3 months
	Polystictus versicolor ...	2 years	4 years
	„ hirsutus ...	3 years	5 years 3 months
Agaricineae	Trogia crispa ...	4 months	1 year
	Schizophyllum commune	6 years 3 months ...	———
	Lenzites betulina ...	3 years	5 years
	Marasmius oreades ...	6 weeks	3 months

Paul Becquerel* has endeavoured to find out whether or not there may be an entire suspension of vitality in the reproductive bodies of plants. He first found that dried seeds of various kinds such as peas, on being placed in pure and dry nitrogen for a year, did not liberate a trace of carbon dioxide and yet germinated subsequently. He† then showed that grains of Wheat and that seeds of White Mustard and of Lucerne, after having their coats perforated, can be subjected to total desiccation in a vacuum of one five-hundredth of a millimetre pressure of mercury for one year, and that during this period they can be subjected for three weeks to the temperature of liquid air (-190° C.), and for seventy-seven hours to the temperature of liquid hydrogen (-253° C.), and that, nevertheless, when subsequently they are supplied with air and moisture at a suitable temperature, they germinate in a normal manner. Becquerel came to the conclusion that his experiments, along with those of his predecessors, indicate that at least for the seeds upon which he experimented, an interruption of life is not only possible but actual, without there being any indication of a limit to its prolongation.

Paul Becquerel‡ has extended his investigations to the spores of certain Moulds: *Mucor Mucedo*, *M. racemosus*, *Rhizopus niger*, *Sterigmatocystis nigra*, and *Aspergillus glaucus*. The spores were dried slowly, in the presence of caustic baryta, for two weeks at 35° C. The containing tubes were afterwards sealed to a Berlement mercury pump and evacuated as perfectly as possible; they were sealed off after a McLeod gauge had indicated a pressure of less than one thousandth of a millimetre of mercury. The vacuum was maintained for twenty-five months, and during this period the spores were subjected to the temperatures of liquid air and liquid hydrogen for three weeks and seventy-seven hours respectively, as was done with the seeds. When the spores were subsequently placed upon sterilized nutritive media, they soon germinated in a normal manner and a fresh crop of spores was quickly produced.

Mr. A. T. Cameron and I,§ working in conjunction, have endeavoured to find out whether or not the fruit-bodies of xylo-

* Paul Becquerel. "Sur la nature de la vie latente des graines et sur les veritables caractères de la vie," Compt. rend., 1906, T. 143, pp. 1177-9.

† Paul Becquerel "Sur la suspension momentanée de la vie chez certaines graines." Compt. rend., 1909, T. 148, pp. 1052-4.

‡ Paul Becquerel, "Recherches expérimentales sur la vie latente des spores des Mucorinées et des Ascomycètes," Compt. rend., 1910, T. 150, pp. 1437-9.

§ *cf.* Buller and Cameron, "On the Temporary Suspension of Vitality in the Fruit-bodies of Hymenomycetes." Read before the Royal Soc. of Canada, May 1912, now in course of publication in the Trans. Roy. Soc., Canada.

philus Hymenomycetes can have their vitality temporarily suspended,—whether they can successfully retain their vitality under conditions which would seem to prevent all metabolism from being carried on. Following, as far as our apparatus permitted, the methods of Paul Becquerel, we observed that the fruit-bodies of *Schizophyllum commune*, after previous thorough drying by exposure to phosphorus pentoxide *in vacuo*, retain their vitality after being kept for sixteen and a half months in a vacuum at a pressure of not more than one-tenth of a millimetre of mercury, in the dark at room temperatures. We also showed that the fruit-bodies, either when moist or dry, are not killed by several hours exposure to a temperature of -35° C., such as occurs during some of the winter nights in Manitoba. These observations pointed to the conclusion that in their retention of vitality, when dried, exposed to ordinary air or kept *in vacuo*, the fruit-bodies of certain Hymenomycetes resemble the seeds of the Higher Plants and the spores of Moulds. Unfortunately, however, the University of Manitoba does not possess a liquid-air plant, so that we were unable to obtain such low temperatures as those used by Becquerel.

Through the kindness of Professor J. H. Poynting, the writer, in the summer of 1912, was supplied with liquid air in the Physics Department of the University of Birmingham. The following experiment was then carried out. Some *Schizophyllum* fruit-bodies which had been already kept dry in ordinary air for two years and eight months, were placed in glass tubes closed at one end. The tubes were then sealed on to a vacuum apparatus consisting of a charcoal-bulb, a phosphorus-pentoxide tube, and an electric-discharge tube. A preliminary exhaustion was made with a Fleuss pump to about 4 mm. of mercury and then the apparatus was sealed off from the pump. The charcoal-bulb was then immersed in liquid air for twenty-four hours to complete the exhaustion, and during this time the fruit-bodies were drying in the presence of phosphorus pentoxide. At the end of this time the discharge-tube showed green fluorescence, proving that the vacuum was exceedingly high,—at about the X-ray stage. The tubes containing the fruit-bodies were then sealed off, removed, and immersed in liquid air contained in a large Dewar flask. Now, therefore, the fruit-bodies which had been thoroughly dried and placed in a very high vacuum, were subjected to a temperature of -190° C. The tubes were kept immersed in liquid air continuously for three weeks. At the end of this time, they were taken out of the liquid air, allowed to take on the room temperature, and then broken open. The fruit-bodies were taken out of the tubes, placed in a damp chamber with wet cotton wool on their upper surfaces, and left for about twelve hours. They were

then placed on a glass slide with their gills turned downwards. In a few minutes, a well-marked spore-deposit collected on the slide, the white lines of which indicated that all the gills were actively discharging spores. In the course of a few hours, a heavy spore-deposit collected, a conclusive proof that the fruit-bodies had resumed their normal activity.

The above experiment has shown that the fruit-bodies of *Schizophyllum commune*, after having been kept dry and exposed to air for two years and eight months, are able to retain their vitality when subsequently they have been dried *in vacuo* by the phosphorus-pentoxide and charcoal-bulb liquid-air method and subjected to the temperature of liquid air for three weeks.

It is difficult to imagine how any metabolism can go on in a fruit-body which is thoroughly dried, placed in a high vacuum, and subjected for three weeks to a temperature of -190° C. The experiment seems to indicate that here, as in the case of Becquerel's seeds and spores, there must be a temporary suspension of vitality. It is possible to conceive of a clock so constructed that it would stop when the temperature of the surrounding air fell below a certain point, or when it was placed in a vacuum, but which would start again when normal conditions were allowed to supervene. Perhaps the machinery of metabolism in the fruit-bodies ceases to work under the conditions of our experiment, but is uninjured and ready to resume its normal course when allowed to do so by a rise in temperature and access to moisture and air. At present I am unable to think of any valid reason why one should not admit the occurrence of a temporary suspension of vitality.